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2. Patent application number *(The Patent Office will fill in this part)* — 0307751.8 — 3 APR 2003

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

University College London
Brook House
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Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — GB

7986/2008

4. Title of the invention — SELF-ALIGNING TISSUE GROWTH GUIDE

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

MEWBURN ELLIS
York House
23 Kingsway
London WC2B 6HP

Patents ADP number *(if you know it)* — 109006

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6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number			

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Date: 2 April 2003

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DUPLICATE

Self-Aligning Tissue Growth Guide

The present invention relates to artificial guides which facilitate the growth of tissues, such as nerves, and may, for example, be surgically implanted into an individual to facilitate the repair of damaged tissue repair.

Damaged tissue within the body is usually repaired by natural processes of regeneration. However, in certain circumstances, the regeneration of damaged tissue is either limited or does not occur at all. Damage to nerves in the peripheral (PNS) or central nervous system (CNS), for example, following trauma or surgery, often results in the permanent loss of sensitivity or function.

Artificial guides have been developed to facilitate the regeneration of neural tissue in both the CNS and PNS. Tubes made from collagen (Labrador et al (1998) Exp. Neurol. 149 243-252) hyaluronan or polylactone have been used to provide global guidance to the neurite outgrowth and isolate the repair region by virtue of the tubular structure. Alternatively, bundles of aligned fibres made from carbon filaments (Khan et al (1991) Brain Res 541 139-145), nitrocellulose paper (Houle et al (1989) Neurosci Lett 103,17-23), collagen (Liu et al (1997) Neurosci Res 49 425-432; Yoshii and Oka, (2001) J. Biomed. Materials Res. 56 400-405) or fibronectin (Priestley et al (2002) J. Physiol-Paris 96 123-133) have also been used to provide contact guidance at a cellular level (reviewed by Brown, (2000) Bioartificial Implants: Design and Tissue Engineering in Structural Biological Materials, design and structure property relationships (Ed M. Elices) Pergamon Materials Series Vol. 4 151-160).

Artificial guides may, for example, be implanted into a site of nerve damage such that the ends of the guide contact the proximal and distal stumps of the damaged nerve. The regenerating nerve grows from the proximal stump into the proximal end of the guide and then through the guide to contact the distal nerve stump at the distal end of the guide and eventually to re-establish a neural connection.

Artificial materials may be used in a nerve guide either alone or with the addition of growth factors (Whitworth et al (1995) Eur. J. Neurosci. 7:2220-2225). Seeding of implants with neural repair cells, which produce growth factors, e.g. Schwann cells, is known to facilitate nerve regeneration (Rodriguez et al (2000) Exp. Neurol. 161 571-584).

Although artificial implants have produced some encouraging results, no functional regeneration of nerves of either the central (CNS) nervous system has yet been achieved following trauma or injury.

The present inventors have produced a guide for tissue growth that is seeded with cells from the tissue. The guide is arranged such that a uni-axial mechanical tension is generated internally within the guide. This tension auto-aligns the cells in the direction of tissue growth to provide a cellular guidance substrate for the regenerating tissue.

One aspect of the invention provides a tissue growth guide for tissue growth comprising,

an inner core comprising a biopolymer matrix having one or more tension generating cells disposed therein,

the guide further comprising an outer sheath surrounding said inner core,

said inner core being fixed to said outer sheath at a first attachment region and a second attachment region;

such that, in use, the cells in said matrix generate mechanical tension in the core between the first and second attachment regions.

Cells within the core produce a contractile force. As the core is fixed to the sheath at the first and second attachment regions, the contractile force generates a tensional load within the matrix. This mechanical tension load is largely co-axial and runs parallel to the direction of tissue re-growth (i.e. longitudinally through the core). The first attachment region is preferably at or adjacent the proximal (entry) end of the guide and the second attachment region is preferably at or adjacent the distal (exit) end of the guide, although in some embodiments the outer sheath may extend beyond the core at each end, for example to facilitate contact with the tissue stumps flanking the damaged region.

The presence of mechanical tension within the surrounding matrix causes the cells within the matrix to align co-axially along the core, i.e. in the direction of regrowth. The tension in the core may also cause the fibres of the matrix to move into a comparable alignment.

Cell level guidance via alignment of extracellular matrices optimises cell migration and is therefore advantageous in a tissue repair guide (Ahmed & Brown (1999) Cell. Motil. Cytoskeleton 42 331-343; Priestley et

al (2002) J. Physiol. Paris 96 123-133; Wojciak-Stothard et al In Vitro Cell Dev. Biol. Anim. 33 110-117).

Tissues suitable for repair as described herein may regenerate or re-grow in a uni-directional manner from one damaged tissue end to the other (e.g. a nerve) or in a bi-directional manner from both damaged tissue ends (e.g. non-neural tissue such as tendon, ligament, meniscus, blood vessel, skin, digestive tract and bone).

Suitable tissues include muscles (in particular neuro-muscular junctions), blood vessels, tendons, ligaments, capsules, meniscus, bones skin and nerves. Some preferred embodiments are directed to the repair of nerves and neural tissue.

The inner core of the guide is preferably linear i.e. it is significantly larger in one dimension than in the other two, and is conveniently rod shaped i.e. it has a round, for example circular or elliptical, cross-section. In embodiments in which the guide is adapted for implantation, the inner core may have a size (i.e. diameter) and length appropriate to connect different tissues and anatomical regions. For example, an inner core suitable for connecting the digital nerve may be about 1mm diameter. Other cores suitable for repair of human nerves may be from 2-7mm diameter.

The biopolymer matrix of the inner core may be a protein-based fibrillar substrate, preferably self-gelling, which is compliant and contractable by the forces generated by embedded cells. Suitable materials include collagen, fibrin/fibrinogen, fibronectin, gelatin, and biosorbable

polymers such as polylactide, polyglycolic acid, and polycapryolactone.

A suitable collagen matrix may be a gel formed of reconstituted network of entangled collagen fibrils of 20-500nm diameter, typically comprising 90%-99% interstitial liquid, depending on the collagen source and reconstitution method.

Suitable collagen matrices include collagen type I matrices which may conveniently be prepared as described in Mudera V.C. et al Cell. Motil. Cytoskeleton (2000) 45 1-9.

A cell suitable for use in the invention applies a contractile force to the biopolymer matrix and falls into alignment with the resultant tension in the matrix. Preferred cells also facilitate growth of tissue within the matrix, for example, by producing one or more appropriate growth promoting factors. For example, a cell suitable for use in the growth of neural tissue (i.e. a neural repair cell) may produce one or more neural growth promoting factors. Neural growth promoting factors include neurotrophins such as neurotrophin-3, nerve growth factor (NGF), glial growth factor (GGF) and brain derived neurotrophic factor (BDNF).

Suitable cells for use in seeding the matrix include Schwann cells, neural fibroblasts, fibroblasts, tenocytes (osteoblasts), myoblasts, smooth muscle cells and endothelial cells. Cells suitable for neural repair include Schwann cells, neural fibroblasts and mixtures thereof (Hall S (2001) J. Hand Surg. 2613 (2) 129-136). Neural repair cells may be obtained from adult nerves by

collagenase digestion or explant culture, as described in 'Neural Cell Culture, a practical approach' Ed Cohen & Wilkin 221-236 IRL Press.

In some embodiments, in addition to tension generating cells (e.g. fibroblasts), the matrix may also be seeded with cells from the tissue of interest. For example, tenocytes, endothelial or epithelial cells, secretory or gland vessels (e.g. sebaceous, pancreatic islet cells, adrenal cortex cells), melanocytes, Schwann cells or astrocytes may be used. In such embodiments, the core containing the aligned tension-generating cells forms a guidance substrate for the growth of the embedded tissue cells.

Cells may be seeded within the matrix by mixing them with the liquid biopolymer matrix and then allowing the liquid matrix to solidify into a gel. Conveniently, the gel may be seeded with 10^4 to 10^7 cells per ml, more preferably $2x10^5$ to 10^6 cells per ml.

The outer sheath is preferably a solid material that provides resistance to the contractile force imparted by the cells in the core, thereby maintaining mechanical tension within the core between the two attachment regions. The outer sheath material is therefore stiff relative to the core biopolymer matrix, in order to accommodate mechanical tension in the core. The sheath material preferably shows low adhesion or substantially no adhesion with the core outside the attachment regions. Preferably, the sheath fully surrounds the core, such that only the ends of the core are exposed to the exterior.

Suitable sheath materials have high biocompatibility i.e. do not produce adverse reactions within the body, and in preferred embodiments are resorbable *in situ*, for example biodegradable, in order to avoid the need for surgical removal from the application site after use. Examples of suitable sheath materials include phosphate glass, polylactone, polyglycone, polycapryolactone and hyaluronan or derivatives thereof. Other suitable materials include collagen, fibrin, fibronectin, cellulose, chitosan, and starch. Suitable non-resorbable sheath materials include silicone.

If the core is a non-protein polymer, the proximal end of the sheath may comprise a protein aggregate material to allow the core to be preferentially released from the proximal end, mediated by cell proteases from the incoming tissue (e.g. a regenerating nerve).

The outer sheath is fixed to the inner core at the first attachment region and the second attachment region such that movement, particularly axial or longitudinal movement, of the core relative to the sheath is prevented in these regions. To allow the generation of a contractile force, the core is preferably free to move relative to the sheath between the first and second attachment regions. To prevent adhesion of the core to the sheath outside the defined attachment regions, the sheath material is preferably non-adherent.

The core and sheath may be fixed together in the attachment regions by any convenient method and the skilled person is able to identify a number of suitable techniques.

In some embodiments, the outer sheath may be mechanically fixed to the core at the first and second attachment regions.

For example, at the first and second attachment regions, the outer sheath may be shaped to provide a cooperative engagement with the inner core. The engagement fixes the sheath and core together at these regions. Preferably, the outer sheath comprises one or more openings or protrusions in its inner surface. Openings may extend through the side of the sheath to the outer surface, or may form recesses or niches that do not extend to the outer surface. Suitable openings include slots, pores, grooves, and apertures. For example, the attachment regions of the sheath may comprise porous cuffs around the core.

Preferably, the core engages with the openings or protrusions in the sheath when it is introduced into the sheath in liquid form during production of the guide. When the core solidifies, the engagement holds the core and sheath together at the attachment regions.

Other mechanical fixing methods, such as pins, sutures, pressure clips or insert clamps, may also be used.

In some embodiments, the outer sheath may be chemically fixed to the core at the first and second attachment regions, for example using an adhesive such as a fibrin or cyanoacrylate adhesive.

As described above, in some embodiments, the guide may be adapted for implantation into an individual to facilitate the repair of damaged tissue.

The outer sheath may, for example, be composed of a non-adherent material which reduces or abrogates the formation of adhesions between the guide and the tissue surrounding the implanted guide (i.e. the sheath is anti- or non-adhesive to surrounding gliding tissue layers). The outer sheath may also reduce or abolish the in-growth of surrounding cells or tissues into the inner core.

The sheath may also be suitable and/or adapted for accepting sutures or other attachment means (e.g. glue) to hold the core against the damaged tissue ends.

A guide adapted for implantation is preferably resorbable *in situ*. More preferably, the stability of the guide (and therefore the resorption rate) varies along its length (e.g. where tissue regenerates in a proximal to distal direction, there may be a gradation in the rate of resorption of the guide between the proximal (entry) and distal (exit) ends and where tissue regenerates from both broken tissue ends (i.e. both ends are proximal), there may be a gradation in the rate of resorption of the guide between the ends of the guide and the middle).

Where tissue regenerates in a proximal to distal direction, for example in nerve repair, the gradation of resorption rate is preferably such that the proximal end of the guide is resorbed more quickly than the distal end. This provides the regenerating nerve end with extended guidance as it passes through the guide. The secretion of cellular factors, including proteases, by the regenerating nerve causes preferential proximal resorption as the nerve enters and grows through the proximal end of the guide.

The resorption of the outer sheath at the first attachment region, after regenerating tissue, such as a nerve, has entered the proximal end of the core, releases the core from the outer sheath at the proximal end. The core is held at its proximal end by the tissue itself and tension previously exerted between the first and second attachment regions is transferred to the regenerating tissue, exerting a traction force. In other words, the outer sheath at the first attachment region is resorbed preferentially after entry of regenerating tissue into proximal end of the core, such that the core applies mechanical tension to the regenerating tissue.

Where tissue regenerates in a multidirectional manner (i.e. from both broken ends of the damaged tissue), the gradation of resorption rate is preferably such that the ends of the guide (which are both effectively proximal ends) are resorbed more quickly than the middle of the guide. This provides the regenerating tissue ends with extended guidance as they pass through the guide and meet within it. This preferential resorption is caused by the release of cellular factors, including proteases, by the regenerating tissue as it enters and grows through the guide.

The resorption of the outer sheath at the first and second attachment regions, after regenerating tissue has entered the ends of the core, releases the core from the outer sheath. The core is held at its ends by the regenerating tissue itself and tension previously exerted between the first and second attachment regions is transferred to the plane of the prospective tissue growth, exerting a traction force. In other words, the

outer sheath at the first and second attachment regions is resorbed preferentially after entry of regenerating tissue into the ends of the core, such that the core applies mechanical tension to the regenerating tissue.

This is advantageous, as the application of mechanical tension to tissue, in particular to nerves, is known to accelerate growth (Smith et al (2001) Tiss Eng. 7 131-139).

Whilst preferential proximal resorption may occur automatically, as the regenerating tissue enters the guide, resorption rate may be further controlled across a guide having a protein-based sheath by soaking one or both ends of the guide in stabilizing reagents such as Cu^{++} or Zn^{++} solutions, to generate a concentration gradient of these ions across the guide, or by fabricating the guide from a number of lengths of guidance material whose adhesion protein composition gradually changes from the proximal to the distal end of the guide.

In use, a guide of the invention may be assembled and optionally, tension in the core allowed to develop prior to implantation, for example by culturing the cells within the core for 8-12 hours. The guide may then be implanted into a site of tissue damage such that the ends of the inner core contact the proximal and distal stumps of the damaged tissue. The guide may be held in place by glue or other fixing means, such as sutures, for example through the outer sheath. The proximal stump of the regenerating tissue, such as a nerve, may enter the guide at its proximal end and exit the guide at its distal end to contact the distal stump. In other embodiments,

regenerating tissue from both broken stumps may enter the guide at its ends and meet within the guide to re-establish a functional connection.

Guides as described herein may be useful in the repair of damage to a variety of tissues, including neural damage in the central or peripheral nervous system, and generation of grafts for plastic surgery.

A tissue growth guide as described herein may be linear and have a first and a second end. In embodiments in which tissue regrowth is unidirectional, the first end may act as a proximal entry port for the regenerating tissue and the second end as a distal exit port. In embodiments in which tissue regrowth is bi-directional, the first and second ends may both act as proximal entry ports for the regenerating tissue.

In some embodiments, the guide may be branched i.e. it may comprise more than two ends, for example, third or fourth ends. Such a branched guide may possess more than one entry port and/or more than one exit port. Preferred embodiments may comprise a single proximal entry port and two or more, for example three, four or five, distal exit ports. A guide according to these embodiments preferably comprises an attachment region at each of its ends to allow mechanical tension to be maintained throughout the core. For example, a first attachment region may be positioned adjacent the proximal entry port and a second and third (or more) attachment regions respectively at the two (or more) distal exit ports.

In other embodiments, a guide of the invention may be adapted for the *in vitro* growth of tissue. Tissue grown

within such a guide may, for example, be subsequently implanted into an individual.

In such embodiments, the cells seeded in the biopolymer matrix may comprise tension generating cells, such as fibroblasts, and additionally cells of the tissue of interest (or progenitor/stem cells capable of differentiating into cells of the target tissue). Suitable tissue cells include tenocytes, endothelial or epithelial cells, secretory or gland vessels (e.g. sebaceous, pancreatic islet cells, adrenal cortex cells), melanocytes, Schwann cells or astrocytes.

A guide seeded with tension generating cells and cells of the target tissue may be cultured in a bioreactor under standard tissue culture conditions (for example, 37°C in DMEM + 10% Foetal Calf Serum) to allow the orientated growth of target tissue cells within the guide.

The guide may be cultured by immersion in culture medium and/or medium may be introduced directly to the interior of the guide by means of capillaries within the core.

The core of a guide according to these embodiments may comprise one or more capillaries for the passage of nutrient medium through the core. Preferably, the one or more capillaries form continuous channels running co-axially along the length of the core.

Capillaries may be introduced to the core during production by conventional techniques such as incorporation and removal of fine suture wire, incorporation of a soluble fibre, introduction of a

chemically degradable layer, or treatment with an optical/radiation source such as a laser.

The one or more capillaries may be connected to a source of nutrient medium. Flow of medium through the capillaries may be induced, preferably by pumping, for example using a peristaltic pump. Flow through the capillaries may be linear, pulsed or cyclical. Medium flowing through the capillaries diffuses through the core to provide suitable growth conditions to the embedded cells.

Two or more tissue growth guides in accordance with this embodiment may be incubated in a common flow controlled bioreactor by connecting an end of the guide to a flow manifold, for example using a luer-syringe type connection, such that nutrient medium flows from the manifold through the capillaries of the core and then on to an outflow.

After growth within the guide, tissue cells may be isolated and or extracted from the guide and used for a variety of purposes, including implantation at a site of tissue damage.

A tissue growth guide as described herein may be produced by introducing the inner core to the outer sheath in a liquid form, so that the sheath moulds the core to the appropriate shape.

An aspect of the invention provides a method of making a guide for tissue growth comprising;

(i) providing an outer sheath,

(ii) introducing cells to a liquid biopolymer matrix,

(iii) introducing said liquid matrix to the interior of the outer sheath,

(iv) causing or allowing said liquid matrix to set; and,

(v) fixing the matrix to the core at a first and second attachment region.

The matrix may be fixed to the core by any one of a range of mechanical or chemical techniques as described above.

Preferably, the sheath and the core are fixed together at the attachment regions through the cooperative engagement of the matrix with the sheath.

A method of making a guide for tissue growth may comprise;

i) providing an outer sheath which is shaped to cooperatively engage the inner core at the first and second attachment regions,

ii) introducing cells to a liquid biopolymer matrix,

iii) introducing said liquid matrix to the interior of the outer sheath such that liquid matrix engages said sheath at the said attachment regions, and;

iv) causing or allowing said liquid matrix to set, such that said engagement prevents co-axial movement of the core relative to the sheath.

The outer sheath may be linear or may have one or more branches e.g. it may be bi- or tri-furcated. The core, which is moulded by the sheath, will naturally adopt the shape of the sheath.

The matrix may be set or solidified by any convenient technique to form the core of the guide, for example incubation at 37°C for 5 minutes; addition or activation of thromin in a fibrinogen containing protein solution (e.g. by adding Ca2+ to plasma fraction); shear aggregation of fibronectin rich protein gels (Brown et al (1994) Biomaterials 15 457-464; Phillips et al (2003) in press), addition of polymerising catalyst to self-setting biodegradable polymers (e.g. Hubbard et al).

Suitable biopolymer matrices, cells and outer sheaths are described above.

In other embodiments, the seeded biopolymer matrix may be introduced to the outer sheath in a non-liquid i.e. gel form and then fixed at the first and second attachment regions. For example, the matrix may be inserted into a tubular outer sheath or the sheath may be wrapped around or applied to the matrix (e.g. by casting a catalysed setting sheath material around the core (e.g. a fibrin rich material or self-setting biodegradable polymer)). The sheath may then be fixed in place. Suitable fixings prevent the axial movement of the core relative to the sheath and may include adhesives, pins, clamps and pressure clips.

A method of producing a tissue growth guide may comprise the further step of; (v) causing or allowing the cells within said matrix to generate mechanical tension between the first and second attachment regions.

Mechanical tension may be generated in the core by culturing the cells in the matrix in appropriate conditions, for example, by placing the guide in a

standard cell culture media, such as DMEM, for example in a petri dish, and incubated for 8 to 12 hours at 37°C. The cell culture medium may optionally be supplemented with ascorbate, to facilitate contraction.

In some preferred embodiments, the guide may then be implanted into a human or animal body for the repair of damaged tissue. For example, the proximal end of the guide may be attached to the proximal stump of a damaged nerve and the distal end of the guide may be attached to the distal stump of a damaged nerve. A tissue growth guide may optionally be implanted without a pre-tensioning step as described above. Mechanical tension is then generated in the core *in situ*.

In other preferred embodiments, the one or more tissue repair cells in the biopolymer matrix may comprise fibroblasts or other tension generating cells, as described above, and additionally cells of the tissue of interest (or progenitor/stem cells capable of differentiating into target tissue cells). Suitable tissue cells include tenocytes, endothelial or epithelial cells, secretory or gland vessels (e.g. sebaceous, pancreatic islet cells, adrenal cortex cells), melanocytes, Schwann cells and astrocytes.

A method according to such embodiments may comprise, after mechanical tension has been generated in the core by the tension generating cells, the step of culturing the tissue cells in said guide. Cells may be cultured by the addition of nutrient medium and appropriate conditions, as described above.

After culturing, the cells in said core may be isolated and or extracted from the guide, for example, for use in therapy, according to standard techniques. In other embodiments, the core or the guide containing the cultured tissue cells may be used directly in therapy, for example implantation for the repair of damaged tissue.

Other aspects of the invention provide a guide as described herein for use in a method of repairing tissue damage, in particular neural damage, and a method of repairing tissue damage to an individual comprising implanting a guide as described herein into said individual.

Implanting may comprise attaching or fixing the guide to the broken ends of a damaged tissue (e.g. the proximal and distal stumps of a damaged nerve), for example using sutures.

Another aspect of the invention provides a method of repairing tissue damage comprising attaching the proximal end and distal ends of a guide as described above to the broken ends of a damaged tissue in an individual (e.g. the proximal and distal stumps respectively of a damaged nerve).

Another aspect of the invention provides a kit comprising a guide for tissue growth as described above or for the production of a guide for tissue growth as described above. A kit may comprise a biopolymer matrix, for example in a solid or liquid form, an outer sheath and one or more tension generating cells and/or cells from a tissue of interest.

Suitable biopolymer matrices, outer sheaths and neural repair cells are discussed above.

The matrix may be a ready prepared gel pre-shaped into the appropriate core shape or may be in a powder or liquid form for moulding into the appropriate core shape using the outer sheath.

The outer sheath may be in the form of a tube which surrounds the inner core, or into which the inner core can be introduced. Alternatively, the outer sheath may be in the form of a flat sheet that is wrapped around or applied to the core prior to the development of pre-stress in the core and implantation.

A kit may comprise one or more additional components such as suturing equipment or glue for fixing the guide to the damaged tissue ends, additional growth factors for incorporating into the inner core and instructions for use.

Aspects of the present invention will now be illustrated with reference to the experimental exemplification below, by way of example and not limitation. Further aspects and embodiments will be apparent to those of ordinary skill in the art.

It will be understood by those of ordinary skill in the art that any combination of the preferred feature that are described in this specification may be used in any combination in accordance with the invention.

All documents mentioned in this specification are hereby incorporated herein by reference.

Experimental

Self-Alignment *in vitro*

A collagen gel was prepared by mixing 2 ml of 2.03mg/ml collagen type 1 (rat tail) with 0.25ml 10xDMEM and neutralising with 5M NaOH. The gel was then added to a 0.25ml cell suspension (mixture of Schwann cells and fibroblasts from primary culture of neonatal rat sciatic nerve explant; 250 000 cells per ml) to produce 2.5ml of cell-seeded gel.

The liquid gel was added to a rectangular mould containing a tethering bar at each end so that the liquid gel became enmeshed with the tethering bars. The liquid gel was then allowed to set by incubation at 37°C for 5 minutes, so that the rectangular gel was anchored at each end by a tethering bar.

The gel was cultured overnight in DMEM + 10% foetal calf serum at 37°C.

After incubation, the gel strip was observed to have changed shape, becoming narrower. Cells within the contracted gel were observed to show alignment, whereas cells in non-tethered control experiments did not show alignment.

Preparation and Implantation of Nerve Guides

8 holes of 0.5 mm diameter are made around each end of a 2mm diameter silicone tube ('Silastic'; Dow Corning) using a 25-gauge needle.

A collagen gel is prepared and seeded with a mixture of Schwann cells and fibroblasts as described above. 200µl of seeded gel is inserted into the tube to fill the tube and mesh with the ends by flowing through the holes.

The gel is allowed to set in an incubator at 37°C for 5 min, placed in a petri dish containing DMEM + 10% FCS and incubated at 37°C overnight. During this incubation, the gel is observed to contract within the tube i.e. spaces formed between the gel and the tube walls, whilst the gel remained tethered at its ends. This results in a thin strand of collagen containing aligned Schwann cells and fibroblasts running down the centre of the tube, held in place at the ends of the tube

The guide may be frozen and sectioned for staining to monitor the cellular composition. Cells in the main part of the gel are orientated axially. Those at the ends aligned with the forces generated by the anchoring of the gel to the tube. Staining the gel construct with haematoxylin and eosin showed the cells, and a phase contrast image of an unstained section from the same construct showed its position in the tube.

After the gel core of the device contracts within the tube overnight, it may be implanted into an animal model of nerve repair. The nerve stumps from a transected sciatic nerve are placed into the ends of the tube up against the ends of the gel and may then be sutured in place.

Claims:

1. A tissue growth guide comprising,

an inner core comprising a biopolymer matrix having one or more cells positioned therein,

the guide further comprising; an outer sheath surrounding said inner core,

said inner core being fixed to said outer sheath at a first attachment region and a second attachment region;

such that said cells produce mechanical tension in said core between the first and second attachment regions.

2. A guide according to claim 1 wherein said the mechanical tension in said core causes alignment of the cells.

3. A guide according to claim 1 or claim 2 wherein said the mechanical tension in said core causes alignment of the fibres of said biopolymer matrix.

4. A guide according to any one of the preceding claims wherein the biopolymer matrix is a collagen matrix.

5. A guide according to any one of the preceding claims wherein said cells comprise one or more of Schwann cells, neural fibroblasts, fibroblasts, tenocytes, astrocytes, osteoblasts, myoblasts, melanocytes, smooth muscle cells, secretory or gland vessel cells, epithelial cells and endothelial cells.

6. A guide according to any one of the preceding claims wherein said sheath is biosorbable.

7. A guide according to claim 6 wherein said sheath is selected from the group consisting of silicone, phosphate glass, polylactone, polyglycone, polycapryolactone, hyaluronan or derivatives thereof, collagen, fibrin, fibronectin, cellulose, chitosan, and starch.

8. A guide according to claim 7 wherein the mechanical tension in the core imparts a traction force on regenerating tissue in the guide.

9. A guide according to any one of the preceding claims wherein the sheath is mechanically fixed to the core at the first and second attachment regions.

10. A guide according to claim 9 wherein said outer sheath is shaped to cooperatively engage the inner core at the first and second attachment regions to prevent co-axial movement of the core relative to the sheath.

11. A guide according to claim 10 wherein said sheath comprises one or more openings which cooperatively engage the inner core at the first and second attachment regions.

12. A guide according to claim 11 wherein said openings comprise a plurality of pores.

13. A guide according to claim 12 wherein said openings comprise one or more holes in the sheath.

14. A guide according to any one of claims 1 to 8 wherein the sheath is chemically fixed to the core at the first and second attachment regions.

15. A guide according to any one of the preceding claims adapted for use as an implant in the repair of damaged tissue

16. A guide according to claim 15 adapted for the regeneration of nerves.

17. A guide according to any one of claims 1 to 14 adapted for *in vitro* use as a bioreactor for the growth of tissue

18. A method of making a guide for tissue growth comprising;

a) providing an outer sheath,

b) introducing cells to a liquid biopolymer matrix,

c) introducing said liquid matrix to the interior of the outer sheath,

d) causing or allowing said liquid matrix to set; and,

e) fixing the matrix to the core at a first and second attachment region.

19. A method according to claim 18 wherein the sheath is shaped to cooperatively engage the inner core at the first and second attachment regions such that the liquid matrix engages said sheath at the said attachment regions, said engagement preventing co-axial movement of the core relative to the sheath when the matrix is set.

20. A method according to claim 18 or claim 19 comprising causing or allowing the cells within said matrix to generate mechanical tension between the first and second attachment regions.

21. A method according to any one of claims 18 to 20 comprising implanting said guide into a human or animal body.

22. A method according to any one of claims 18 to 21 wherein the cells comprise fibroblasts and one or more cells of said tissue.

23. A method according to any one of claims 18 to 21 wherein the tissue regeneration cells comprise fibroblasts and one or more stem cells or progenitor cells of cells of said tissue.

24. A method of repairing tissue damage comprising linking a first and a second end of a guide according to any one of claims 1 to 16 to the broken ends of a damaged tissue in an individual.

25. A method according to claim 24 wherein the damaged tissue is a nerve.

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